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Whitefly DNA extraction, partial mtCO1 gene amplification and gel electrophoresis

In 2 collections

Joachim Nwezeobi¹, Onyeyirichi Onyegbule², Chukwuemeka Nkere², Joseph Onyeka², Sharon van Brunschot³, Susan Seal³, John Colvin³

¹National Institute of Agricultural Botany, Cambridge, United Kingdom;

²National Root Crops Research Institute, Umudike, Umuahia, Abia State, Nigeria;

³Natural Resources Institute, University of Greenwich, Central Avenue, Chatham Maritime, Kent, United Kingdom

Joachim Nwezeobi

MATERIALS

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- ⊗ Gel Loading Dye Orange (6X) - 4.0 ml **New England Biolabs Catalog #B70229**
- ⊗ Chelex 100 **Sigma Aldrich Catalog #C7901-100G**
- ⊗ 70% ethanol **Fisher Scientific**
- ⊗ TBE buffer (Tris-Borate-EDTA), 5x Solution **Bio Basic Inc. Catalog #A00265.SIZE.4L**
- ⊗ PCR-Grade Water **Sigma Aldrich Catalog #W3500**
- ⊗ Agarose **Thermo Fisher Catalog #75510019**
- ⊗ GeneJET PCR Purification Kit **Thermo Fisher Catalog #K0701**

- 1 Single whitefly adults were randomly chosen from each of the 1.5 ml Eppendorf tubes containing 70% ethanol, using a sterilized entomological pin.

- 2 Each whitefly was transferred to a clean 1.5 ml Eppendorf tube.
- 3 To extract insect DNA, 50 µl of 10% Chelex was added to the tube and the whitefly was crushed using a plastic pestle until a clear homogenised mixture was obtained.
- 4 The mixture was then incubated at 56°C for 20 minutes and further incubated at 100°C for five minutes.
- 5 The mixture was then centrifuged at 13,500 g for five minutes using the Eppendorf Centrifuge 5424 R and the supernatant was collected and stored at -20°C until use.
- 6 Two sets of primers, with different degrees of sensitivity, were used to amplify the mtCO1 fragments, which were the: (i) African specific 2195Bt (5'-TGRTTTTTTGGTCATCCRGAAGT-3') and C012/Bt-sh2 (5'-TTTACTGCACTTTCTGCC-3') primers [21] and (ii) generic CI-J-2195 (5'-TTGATTTTTTGGTCATCCAGAAGT -3') and TL2-N-3014 (TCCAATGCACTAATCTGCCATATTA -3') primers.
- 7 Polymerase chain reaction (PCR) was run on an Applied Biosystems 2720 thermal cycler for 35 cycles.
- 8 The PCR program conditions were set at (i) initial denaturation at 94°C for 2 minutes, (ii) denaturation at 94°C for 20 seconds, (iii) annealing at 52°C for 30 seconds, (iv) extension at 72°C for one minute and (v) final extension at 72°C for five minutes.
- 9 Agarose gel electrophoresis (1% w/v in 0.5 X Tris/borate/EDTA buffer) was used to confirm PCR amplification.

- 10** The GeneJETPCR purification kit (Thermo Scientific) was used to purify amplicons of interest as described by the kit manufacturer.